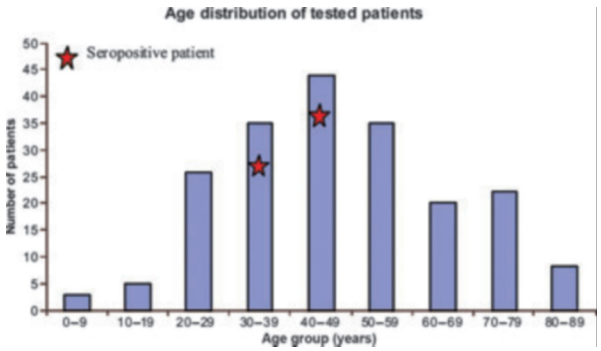

FIG. 1. Age distribution of the 198 patients tested for SFSV IgG.

species is rare, in agreement with the low seroprevalence rates. Moreover, one can argue that these two patients acquired SFSV infection outside of France. One of the two positive patients could be contacted, and he reported several vacation trips to North Africa, during which infection with SFSV could have occurred, although he did not recall fever during or after the trips. As the seroprevalence rates observed with Toscana virus are much higher [9], it is important to underline that there is no cross-reactivity between Toscana virus and SFSV in immunofluorescence assays. Nevertheless, sandflies are common in the south of France, where they are known to be vectors of Toscana virus [9,10], one of the main agents of summer fever and aseptic meningitis in Mediterranean countries. SFSV-like viruses have been recently reported in sandflies other than *P. papatasi* in Algeria and Tunisia [4–6]. In Algeria, 5% of the tested sera contained antibodies reacting against SFSV [6]. Together, these findings raise the possibility that SFSV might be circulating significantly in south-eastern France through sandfly vectors other than *P. papatasi*. The present study does not support this hypothesis.

In conclusion, the low seroprevalence rates observed here for SFSV and SFSV-like viruses, together with the lack of documented cases of infection in the literature, suggest that SFSV is not likely to be of major medical importance in the Marseille area. This remains to be confirmed in other regions of France where sandflies are present.

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Transparency Declaration

Conflicts of interest: nothing to declare.

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Molecular and immunohistochemical detection of rotavirus in urinary sediment cells of children with rotavirus gastroenteritis

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Abstract

This is the first report showing that rotavirus infects the urinary sediment cells in immunocompetent children with rotavirus gastroenteritis. We found that inclusion-bearing cells were frequently detected in the urine samples of patients with rotavirus gastroenteritis. These cells were positive for cytokeratin, which was sometimes coexpressed with rotavirus antigen, in our immunohistochemical analysis. Moreover, in nested RT-PCR experiments, we detected rotavirus double-stranded RNA in some urine samples of patients with rotavirus gastroenteritis. We concluded that rotavirus could lead to infection of the urinary sediment cells concomitantly with rotavirus gastroenteritis.

Keywords: Immunohistochemistry, inclusion-bearing cells, rotavirus gastroenteritis, RT-PCR, urinary sediment cells

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Introduction

Rotavirus (RV) is one of the most common pathogens causing gastroenteritis in young children [1,2]. Acute pre-renal failure following RV-induced dehydration results in high mortality rates in developing countries [1,2]. Recently, some patients with RV gastroenteritis were reported to manifest not only acute pre-renal failure, but also other complications, such as encephalitis, hepatitis, and viraemia [3–5]. However, to date, the role of RV in the development of extra-gastrointestinal infection has not been fully understood [5].

In a case report of fatal RV infection, viral RNA and RV non-structural proteins were detected in several tissues, including the heart, central nervous system, and kidney [6]. However, RV antigen and/or double-stranded RNA (dsRNA) were not detected in tissues, except for the gastrointestinal tract, serum, cerebrospinal fluid, and sputum, of patients with RV gastroenteritis [1,2,7]. In this study, we investigated whether RV infects urinary sediment cells in patients with RV gastroenteritis.

Materials and Methods

Samples

A total of five RV-infected samples were obtained from immunocompetent children at the time of diagnosis of RV gastroenteritis. To ensure no stool contamination, children over 3 years of age who could urinate independently were included in the study. The diagnosis of RV gastroenteritis was confirmed by commercial enzyme immunoassay (Rapid-Testa Rota Adeno; Sekisui Medical, Tokyo, Japan). Samples were also obtained from non-RV gastroenteritis patients whose stools did not show RV antigen on EIA, or other pathogenic bacteria in stool cultures. Negative control samples were obtained from a patient with IgA nephropathy.

Immunohistochemistry of the urinary sediments

First, 10 mL of urine was centrifuged at 500 ***g*** and 4°C for 5 min to separate the urinary sediments from supernatants. Sediments were then washed twice with 10 mL of clean phosphate-buffered saline. Then, urinary sediments were resuspended and fixed with 200 μL of 2% poly(ethylene glycol) and 50% ethanol (Matsunami Glass, Osaka, Japan) for 15 min. After fixation, 50 μL of sediment was placed on a glass slide and dried promptly. The slides were washed with 95% ethanol for 15 min, and blocked with 0.3% hydrogen peroxidase in methanol at room temperature for 30 min. The urinary sediments were stained with mouse anti-human cytokeratin (CK) and anti-epithelial membrane antigen monoclonal antibodies (Dako Japan, Tokyo, Japan) as primary antibodies. They were then visualized with the EnVision system (Dako).

Immunofluorescent antibody technique for staining urinary sediments

With an indirect immunofluorescent antibody technique, urinary sediments were stained by the same procedure as used for immunohistochemistry with the primary antibodies. To visualize the antigen stained with the primary antibodies, we used fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Becton Dickinson, Franklin Lakes, NJ, USA) as secondary antibody. For detection of the RV antigen, we used rabbit anti-RV polyclonal antibodies for VP1, VP2, VP3, VP6, and VP7 (in-house), visualized with rhodamine-conjugated

goat affinity-purified antibody against rabbit IgG (Cappel, MP Biomedicals, Solon, OH, USA). RV-infected MA104 cells (in-house) were used as positive controls.

RT-PCR to detect rotavirus RNA in stool and urine sediments

Thereafter, stools and urinary sediments were analysed with nested RT-PCR to detect viral dsRNA (encoding the VP7 outer capsid protein and serotypes G1, 2, 3, 4, 8, and 9) by use of consensus primers [8]. RNA was extracted with TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). PCR was first carried out with initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 54°C for 1 min, and 72°C for 1.5 min. Multiplex PCR was then carried out for 30 cycles. The PCR products were analysed by electrophoresis on 1% agarose gel containing ethidium bromide, and visualized under UV light.

Results

A small but significant number of inclusion-bearing cells were frequently detected in the urine samples of patients with RV gastroenteritis (Fig. 1a). These cells were positive for CK but negative for epithelial membrane antigen, which indicated that they were of renal tubular epithelial cell origin, similar to the CK-positive cells in the urine of an IgA nephropathy patient (data not shown). Furthermore, we found that about 40% of the CK-positive cells (fluorescein isothiocyanate) coexpressed RV antigens (rhodamine), as represented by the double-positive cells with orange cytoplasm in the merged picture (Fig. 1b).

In nested RT-PCR experiments, a clear PCR product was detectable in stool samples of patients with RV gastroenteritis (Fig 1c, lane 1). RV dsRNA was detectable in four of five patients with RV gastroenteritis, although to a variable degree (Fig. 1c, lanes 2–6). Importantly, no PCR product was detectable when non-RV gastroenteritis or IgA nephropathy samples were used for the assay. Representative samples are shown in Fig. 1c (lanes 7 and 8). The RV dsRNA in the stools and urinary sediments were all of serotype G3 (374 bp), because no PCR products were obtained when primers for other serotypes were used for the assay.

Discussion

This is the first study to find evidence of RV infecting the urinary sediment cells of immunocompetent children with RV gastroenteritis, using urinary sediments from affected patients.

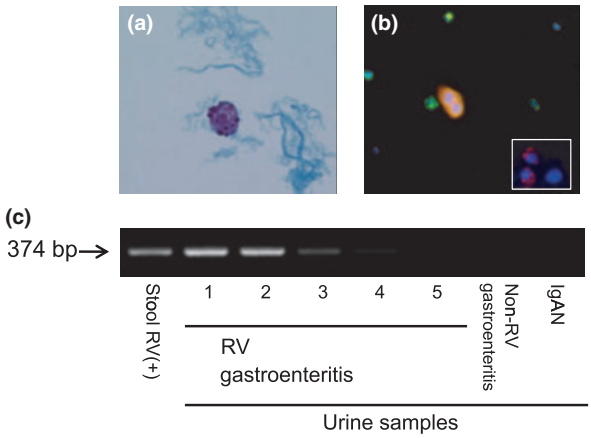

FIG. 1. Detection of rotaviral antigens and double-stranded RNA in urinary sediments of rotavirus (RV)-infected patients. (a) Renal tubular cell-shaped inclusion-bearing cell. (b) Immunofluorescent staining of renal tubular cells: a cytokeratin-positive cell (fluorescein isothiocyanate: green) coexpressing RV antigen (rhodamine: red); a double-positive cell with orange cytoplasm (merged: orange). The cells in the lower box are RV-infected MA104 cells (positive controls). (c) Results of nested RT-PCR of urinary sediments. Four of five RV-infected patients showed RV RNA. The stool of an RV-infected patient was used as a positive control. The urine from a non-RV gastroenteritis patient and an IgA nephropathy (IgAN) patient were used as negative controls.

To confirm RV infection of the kidney, direct analysis with a kidney section is preferable to analysis of urinary sediments. However, it is very difficult to obtain kidney sections, because RV gastroenteritis is always self-limiting, and renal biopsy has ethical implications. We therefore selected urine samples from patients with RV gastroenteritis.

Inclusion-bearing cells were observed in the urinary sediments of RV gastroenteritis patients (Fig 1a). In general, inclusion-bearing cells in the urine indicate viral infection [9]. Taken together, these results suggest that RV infects urinary sediment cells in RV gastroenteritis patients.

We then found cells double-positive for RV antigen and CK (Fig. 1b) in the urinary sediments of RV gastroenteritis patients, indicating RV infection of renal tubular epithelial cells. Moreover, nested RT-PCR detected RV dsRNA in the urinary sediments of RV gastroenteritis patients.

We did not investigate clinical significance in this study, and therefore the relationship of RV kidney infection to RV gastroenteritis remains uncertain. It is also unclear what types of urinary sediment cell are infected by RV in addition to renal tubular epithelial cells. The findings of this study were obtained from a small sample, and therefore they must be confirmed in a larger one. It is essential to conduct further studies to examine the clinical aspects of RV renal infection.

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Transparency Declaration

There are no conflicts of interest to declare.

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